MICROFABRICATED MICRODIALYSIS MICRONEEDLES FOR CONTINUOUS MEDICAL MONITORING

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Abstract – Enzyme based biosensors suffer from loss of activity and sensitivity through a variety of processes. One major reason for the loss is through large molecular weight proteins settling onto the sensor and affecting sensor signal stability and disrupting enzyme function. One way to minimize loss of sensor activity is to filter out large molecular weight compounds before sensing small biochemicals such as glucose. A novel microdialysis microneedle is introduced that is capable of excluding large MW compounds based on size. Preliminary experimental evidence of membrane permeability is shown, as well as diffusion and permeability modeling. Solutions should be able to equilibrate across the microdialysis membrane in a few seconds, as opposed to a few minutes with existing technologies. Microdialysis microneedles present an attractive first step towards decreasing size, patient discomfort and energy consumption of portable medical monitors over existing technologies.

I. INTRODUCTION

Microneedles have emerged as promising micro-fabricated devices that have biological and medical applications [1-5]. Small needles are desirable because they reduce insertion pain and tissue damage in the patient and can be integrated into a variety of devices. They may be used for parallel fluid sampling, analysis, and drug delivery. Microfabricated needles are especially attractive because they may be fabricated with great precision. This precision can provide systems for which penetration depth can be controlled and drug delivery may be enhanced by targeting specific tissue types below the skin. For example, this targeting is important for interstitial fluid sampling, where shallow needle penetration makes it highly unlikely for blood vessels to be severed to corrupt sampling.

When microneedles are used to sample interstitial fluid, then biosensor stability and lifetime can be extended if metabolites are filtered through a microdialysis needle before fluid is moved onto the sensor. Protein adsorption onto a sensor seriously affects the stability and lifetime of a sensor. For this reason, many commercial enzyme based biosensors (e.g. glucose sensors) are single-use systems. In order to accurately monitor biochemical concentrations, single-use sensors must be calibrated against a standard solution prior to use.

Another approach is to protect the sensors from protein adsorption and subsequent loss of enzyme activity by using diffusion membranes. Even with these precautions, sensors are only reliable within 10-15% of the true chemical concentration. Protein adsorption is a critical bottleneck to the development of continuous electrochemical monitors.

Diffusion membranes are also susceptible to protein adsorption which affect the mass transfer characteristics of the sensor. Because electrochemical sensors are usually operated in a diffusion limiting regime, a decrease in mass transfer rate will lead to a decrease in sensor signal. Free enzymes in solution can catalyze side reactions where the products of these reactions increase the noise and false signal at the sensor. Free proteins can also disrupt enzyme function so reactions are not catalyzed and no signal is detected by the sensor.

For example, a glucose sensor uses the enzyme glucose oxidase to catalyze the reaction between glucose and oxygen to produce gluconic acid and hydrogen peroxide (H_2O_2). The H_2O_2 is subsequently oxidized at a platinum electrode to generate a current which is directly proportional to the glucose concentration [6]. If there is a loss of enzyme activity then H_2O_2 is not produced and the signal at the sensor will be lower than the actual glucose concentration. Removing free protein from biological solutions before sensing metabolites will help increase sensor accuracy and lifetime.

Current microdialysis needles have a very large bore, consume a large amount of fluid, and are uncomfortable during insertion and prolonged wear. They also require a serial assembly in which a dialysis membrane is inserted and bound into an 18-gauge needle. There are also dialysis type needles with integrated glucose sensors in the bore of a stainless steel needle which require even more assembly [6]. Dialysis fluid flows at 1 μl/min with a 2 minute equilibrium time [7]. In addition, the large needle is inserted directly into the bloodstream which causes discomfort.

A microfabricated dialysis microneedle (Fig. 1) has been developed which is permeable to small molecular weight (MW) molecules, yet excludes large MW compounds such as proteins. This microneedle, compared to existing technologies, is an order of magnitude smaller in almost all characteristics, while meeting the same performance criteria. It is also fabricated in situ so no assembly is necessary. In addition, the needle can also easily be integrated with planar electrochemical sensors
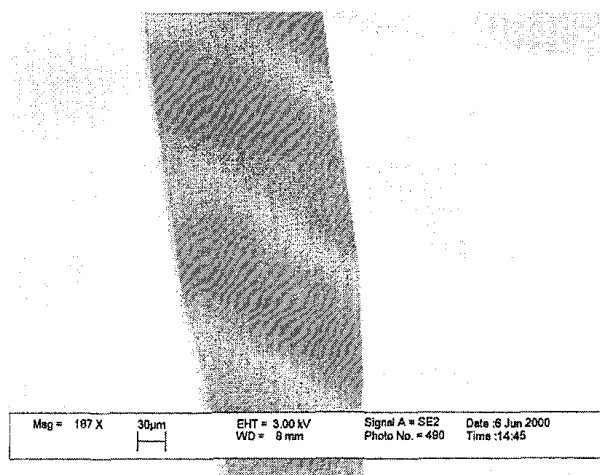
Fig. 1 SEM picture of a microdialysis microneedle. The arrayed dots on the needle represent 2 µm features defining the dialysis membrane.

either by batch transfer or direct fabrication on the needle so no serial assembly with sensors would be required. The fluid channel is only 10 µm high (Fig 2). This dramatic miniaturization results in smaller sample volumes, more rapid diffusion equilibrium of analytes between interstitial fluid and dialysis fluid, and a lower overall power to operate the system. The needle protrudes just below the stratum corneum (the topmost layer of the skin), and analyte concentrations equilibrate between the interstitial fluid and dialysis fluid. The concentration of many analytes in interstitial fluid, including glucose, is strongly correlated with blood concentrations, while the superficial protrusion of the needle under the skin minimizes patient discomfort.

Microdialysis needles are based upon the direct separation of large MW from small analytes through the dialysis membrane. Such approaches have been used for immunoisolation of cells in microfabricated biocapsules [8,9]. However, these biocapsules still allow the diffusion of smaller proteins such as insulin. The microdialysis membranes presented here are expected to have a size exclusion property, so that even if a smaller molecular
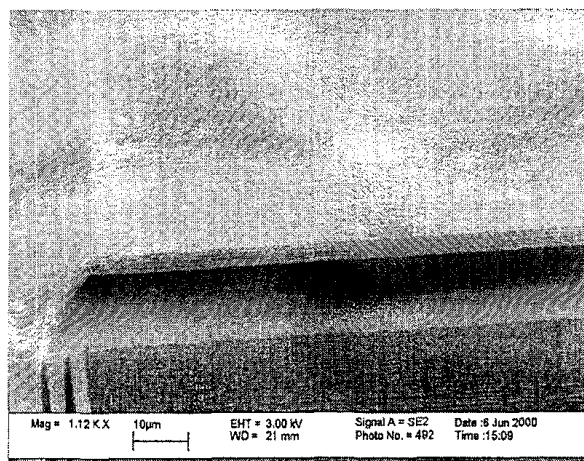
weight protein enters the diffusion channel, its mobility will be retarded by the geometry of the channel.

II. FABRICATION

Needles are fabricated as a polysilicon microshell on top of a silicon substrate [2,5] using a silicon on insulator (SOI) device layer as a mechanical support. Two permeable needle designs have been fabricated (Fig. 3). The first is a diffusion membrane fabricated using a layered polysilicon sandwich with a thin thermally grown oxide (10-50 nm) between the layers, and appropriate etch access holes [8,9]. A second microdialysis membrane takes advantage of permeable polysilicon. Lebouitz [5] used this technology for vacuum encapsulation of resonators. Permeable polysilicon has pore defects (5-20 nm wide) which allow small molecules to permeate between the polysilicon grains.

To fabricate the diffusion membrane 10 µm of sacrificial low temperature oxide (LTO) (450°C, 90 sccm O_2, 60 sccm

Fig. 2. The back of a microneedle showing the microshell fabricated on top of the substrate.
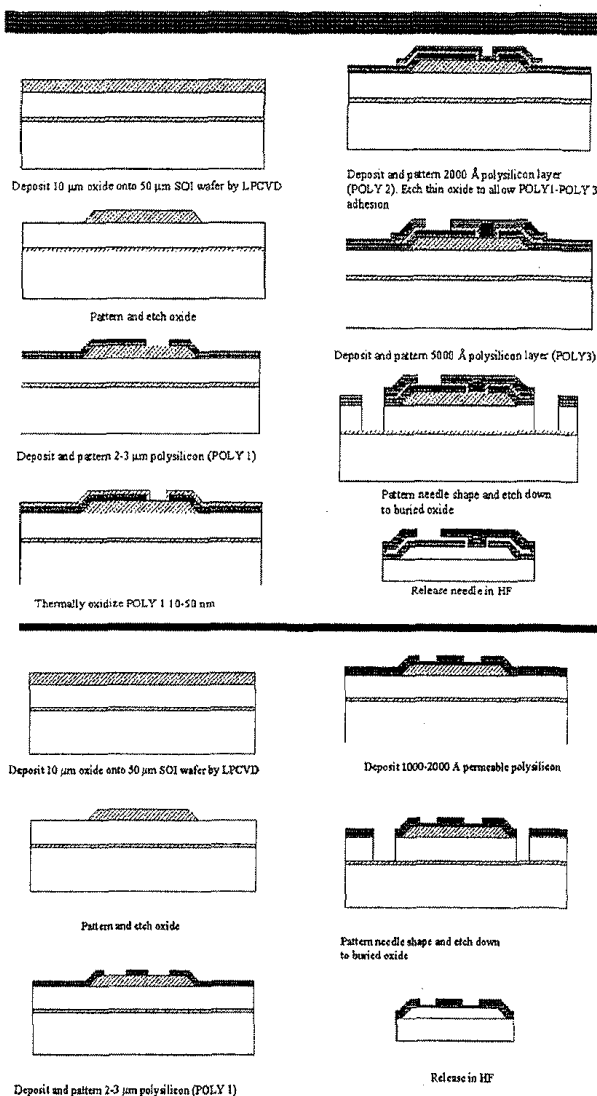

Fig. 3. Fabrication details of the microdialysis membranes. (Top) Diffusion passage type membrane. (Bottom) permeable polysilicon membrane.

SiH$_4$, and 450°C) is first deposited onto a 50 μm thick device layer of a SOI wafer by low pressure chemical vapor deposition (LPCVD). The LTO is annealed at 900°C in a nitrogen atmosphere, masked and etched (CF$_4$ plasma) to define the flow passage. Then 2-3 μm of polysilicon is deposited (100 sccm SiH$_4$, 300 mTorr, 580°C) to define the microshell. The polysilicon is annealed in a nitrogen atmosphere at 900°C to ensure a tensile stress in the film. The polysilicon is masked with 2 μm open holes and etched in a Cl$_2$ plasma. The polysilicon is then oxidized in a dry oxygen atmosphere at 900°C to grow 10-50 nm of thermal oxide which defines the diffusion passage. 200 nm of polysilicon is then deposited and annealed as before. This thin polysilicon layer is masked, etched and adhesion anchors are defined by etching the thin oxide in hydrofluoric acid (HF). A 500 nm polysilicon layer is deposited, annealed, masked and etched to intersect with the thinly grown oxide. The needle shape is then patterned and the needle is etched down to the buried oxide of the SOI wafer in a surface technologies systems (STS) deep reactive ion etcher (DRIE). The needles are released in concentrated hydrofluoric acid for several hours until all the sacrificial oxide is removed. This release also opens up the diffusion channel. Since all the polysilicon has been annealed to be tensile, there should be no buckling between layers which could pinch off the channels (Fig. 4).

The permeable polysilicon membrane is fabricated similarly to the diffusion membrane. First a 10 μm LTO is deposited onto a SOI wafer. Then 2-3 μm of polysilicon is deposited, annealed, patterned and etched. 120 nm of permeable polysilicon is then deposited at 597°C, 125 sccm SiH$_4$, and 555 mTorr. The needle shape is patterned and cut out using the STS. The needle is then released in HF for several hours (Fig. 4).

III. THEORY

A. Fluid Flow

For fully developed flow in the laminar regime the x-directed velocity profile in a rectangular duct with y and z cross section is given by

$$v_x = \frac{16a^2}{\mu\pi^3}\left(-\frac{dP}{dx}\right)\sum_{i=1,3,5,...}^{\infty}(-1)^{(i-1)/2}\left[1-\frac{\cosh(i\pi z/2a)}{\cosh(i\pi b/2a)}\right]\frac{\cos(i\pi y/2a)}{i^3}$$

$$-a \le y \le a$$
$$-b \le z \le b \tag{1}$$

where $2a$ is the height of one of the walls, $2b$ is the width of the other wall, P is the pressure and μ is the viscosity of the solution. Integrating this profile across y and z gives the average flow rate, Q as

$$Q = \frac{4ba^3}{3\mu}\left(-\frac{dP}{dx}\right)\left[1-\frac{192a}{\pi^5 b}\sum_{i=1,3,5,...}^{\infty}\frac{\tanh(i\pi b/2a)}{i^5}\right] \tag{2}$$

The average velocity, U, is $Q/(4ab)$. Microdialysis needles have a 65 μm wide passage and with a height of 10 μm. This may be approximated by plane poiseulle flow.

$$v_x = \frac{a^2}{2\mu}\left(-\frac{dP}{dx}\right)\left[1-\left(\frac{y}{a}\right)^2\right] \tag{3}$$
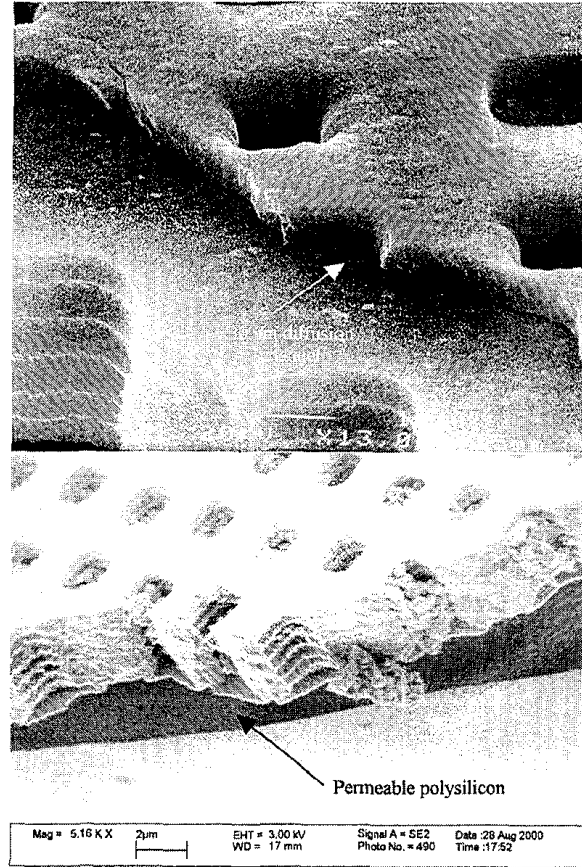
Integrating across y and z gives

Fig. 4. (Top) Diffusion channel dialysis membrane. A thin (30 nm) opening can be seen between the polysilicon layers. In addition, when the layers were fractured, they delaminated and fractured at different points indicating the layers are separate. (Bottom) Permeable polysilicon membrane. The permeable polysilicon layer can be seen at the bottom of the trenches.

$$Q = \frac{4ba^3}{3\mu}\left(-\frac{dP}{dx}\right) \tag{4}$$

Please note that Eqns (3) and (4) also assume a height of $2a$ and a width of $2b$. Comparing (2) and (4) shows the flow resistance to be in agreement within 10 percent.

B. Mass transfer in the microchannel

In order to desciribe the mass transfer characteristics in the channel Fick's second law for single component diffusion is considered.

$$\frac{\partial C}{\partial t} + \vec{u}\cdot\vec{\nabla}C = D\vec{\nabla}^2 C \tag{5}$$

where C is the concentration and D is the diffusivity of the component of interest. For small molecules such as glucose D is assumed to be 10^{-9} m^2/sec. If 1-dimensional unsteady diffusion is assumed, then (5) simplifies to

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial y^2} \tag{6}$$

This can be easily solved by separation of variables with the boundary conditions $C=C_O$ at $y=0$, $t \ne 0$, a no pentration

boundary condition, $\partial C / \partial y = 0$ at $y=2a$ (the height of the wall) for all times, and the initial condition that $C=0$ everywhere at $t=0$, as

$$C = C_O\left[1 - \sum_{r=1,3,5...}^{\infty} \frac{4}{i\pi} \sin\left(\frac{i\pi y}{4a}\right) Exp\left(-\frac{(i\pi)^2 Dt}{16a^2}\right)\right]. \qquad (7)$$

If diffusion out of the needle into the ambient is considered, then the needle can modeled as a constant source diffusing into a semi-infinite medium. The diffusion equation can be solved by a similarity solution

$$C = C_O\left[1 - erf\left(\frac{y}{\sqrt{4Dt}}\right)\right]. \qquad (8)$$

If forced convection from fluid flow in the microchannel is considered, then computational methods must be employed to find the time varying concentration profile with coupled fluid flow.

C. Membrane Permeability

The largest barrier to mass transfer is the dialysis membrane. Since the pore sizes are much larger than the molecules of interest we can define permeability as

$$P_n = \frac{Dkp}{dT} \qquad (9)$$

where k is a partition coefficient (assumed to be 1), p is the porosity or volume fraction of pores versus total membrane volume, and T is the tortuosity of the membrane because the diffusion distance is larger than the thickness of the membrane. For the diffusion channel membrane described with a 30 nm diffusion channel $T=2$ and $p=0.016$.

The time for such a membrane to come to equilibrium is therefore approximated by compartment diffusion as

$$\frac{V}{A_m P_n} = \frac{2a}{P_n} \qquad (10)$$

where V is the volume of the needle, and A_m is the area of the membrane for diffusion. This expression becomes the height of the flow passage divided by the permeability of the membrane.

The dialysis membranes are designed to exclude large molecules based on size. However, many proteins may be less than 30 nm in radius. They should be diluted by a size exclusion effect where steric hindrances (the molecules being the same order of magnitude in size as the flow passages and therefore are retarded by the pores) will slow proteins migration through the thin pores.

The diffusion coefficient for a large component n in a pore can be defined as

$$D_n^* = F(a/r)D_n \qquad (11)$$

where $F(a/r)$ is the centeline approximation as described in [10]

$$F(a/r) \approx 1 - 2.1044(a/r) + 2.089(a/r)^3 - 0.948(a/r)^5$$
$$a/r < 0.4$$
$$(12)$$

and D_n is approximated by the Stokes-Einstein relation

$$D_n = \frac{kT}{6\pi a\mu} \propto M^{-1/3} \qquad (13)$$

where M is the molecular weight of the molecule. As the molecular wieght of a molecule increases, its diffusion coefficient is smaller causing slower migration.

IV. EXPERIMENTAL APPARATUS

To test the permeability of microdialysis microneedles, a concentrated fluorescein solution was visualized diffusing out of the needles. The needle was packaged by placing its two ends (the flow passage is U shaped) in polyimide tubing. The tubing is then sealed using a two part epoxy (Fig. 5). Once the epoxy is dried, flexible tubing is placed inside the polyimide tubing and sealed using heat shrink tubing. A stainless steel needle is attached to the tubing and dye solution is infused through the needle at varying flow rates. The dye is visualized on a Olympus IX70 microscope with a mercury lamp light source.

V. RESULTS AND DISCUSSION

In order to predict the effectiveness of a microdialysis needle, the mass transfer characteristics have been modeled in relevant medical situations. Since one of the largest applications of electrochemical sensors is for diabetics most of the modeling is done assuming glucose is diffusing into the needle. Therefore, it is assumed that the needle is bathed in interstial fluid with a constant concentration source of glucose in the physiological range (5 mM).

Membrane permeation has been analyzed. Equation (10) predicts that in a channel with a height of 10 μm, the microdialysis membrane should come to equilibrium in 2.5 seconds. In addition, as shown in Fig. 6, (7) predicts that the dialysis solution in the channel will come to equilibrium in 0.3 seconds if a one dimensional diffusion solution is assumed. However, the largest resistance to mass transfer is the dialysis membrane and not free diffusion of small molecules into dialysis fluid. An upper time bound for the fluid in the needle to come to equilibrium with the outside may be estimated by defining a perturbed diffusivity $D' = Dp/T$. D' for a small molecule such as glucose through the microdialysis membrane is 125 times smaller than its diffusity in water (10^9 m^2/s). Solving (7) by substituting in D' predicts that the channel will come to equilibrium in 15 seconds. This is the maximum time needed for fluid a 10 μm high channel to come to

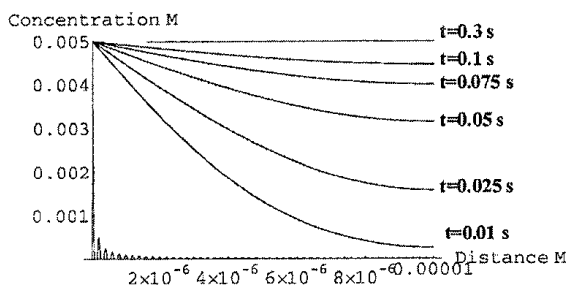Fig. 5. Packaged microneedle next to a dime for size comparison

Fig. 6. Theoretical concentration profile of glucose from a 5 mM constant source. The diffusion constant is assumed to be 10^{-9} m^2/s for small molecules. The diffusion is in the y direction.

equilibrium with the dialysis membrane on top of it.

If it is assumed that plane poiseuille flow (3) is dominating the flow pattern in the channel, (5) can be computationally solved in 2 dimensions (Fig. 7). The solution to the diffusion equation is generated using a computational fluid dynamics package (CFD-Ace, CFD Research Corp.) which accounts for the coupling of the flow profile with the concentration gradient. The fluid is assumed to be moving through the needle at 1 nl/s. 300 µm down the channel, after the entrance region, the solution to (5) in 2 dimensions is very similar to (3), the one dimensional diffusion solution. There is no concentration gradient in the x direction, only in y.

The two-dimensional solution to (5) has also been applied to determine the time to equilibrium through the combined dialysis membrane and flow channel (Fig. 8). The 2 µm membrane is assumed to have diffusivity D' as discussed earlier. Again, 300 µm down the channel in the x direction, the solution becomes essentially a one-dimensional diffusion problem. In addition, it takes about 15 seconds for the channel to come to equilibrium with a 1 nl/s flow rate.

In order to visualize that the diffusion membrane does in fact allow mass transfer across it, fluorescein dye (MW 322) has been visualized diffusing from the needle. These experiments were performed on diffusion passage type
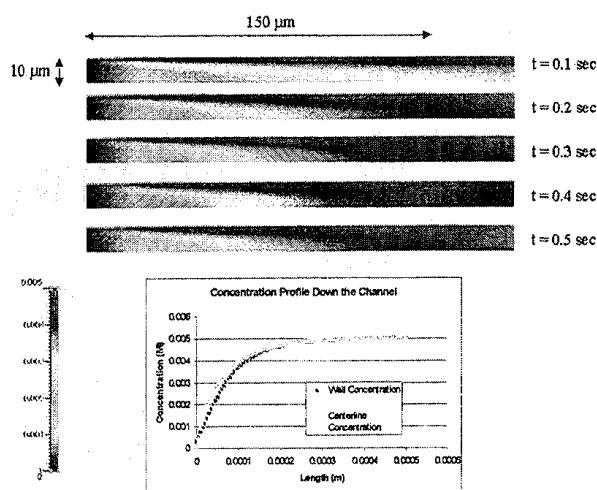

Fig 7. 2-dimensional concentration profile from a constant source with parabolic fluid flow at 1 nl/s. The concentration equilibrates down the channel in less than 1 second.
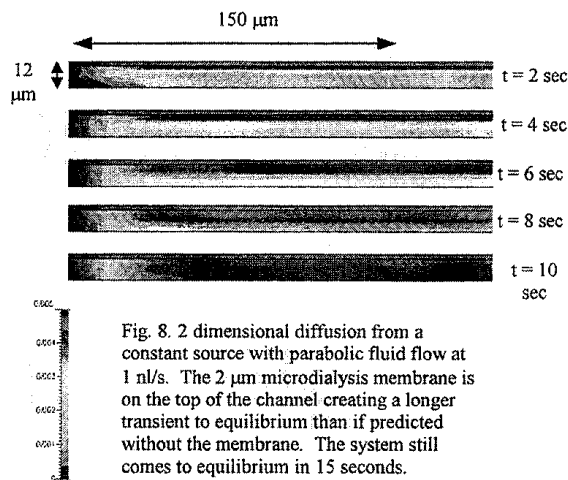

Fig. 8. 2 dimensional diffusion from a constant source with parabolic fluid flow at 1 nl/s. The 2 µm microdialysis membrane is on the top of the channel creating a longer transient to equilibrium than if predicted without the membrane. The system still comes to equilibrium in 15 seconds.

microneedles. First, dye was allowed to come to equilibrium with a droplet of water on the surface of the needle through the membrane. (Fig. 9). The dye can be seen on the outside of the needle and emerging from the needle. Next, the needle was submerged in water and dye was infused at a rate of 10 nl/s and fluorescence profiles were visualized as dye diffused out of and away from the needle (Fig. 10). The fluorescence profile diffusing out of the needle follows the error function profile predicted in (8). These visualizations show that the needle microdialysis membranes will allow mass to permeate.

Large MW proteins are expected to be selectively excluded from the microneedles. In [8] and [9] Desai and co-workers were able to show immunoisolation through similarly sized pores. This implies that a 150 kDa antibody cannot permeate through a pore. However, Desai and co-workers were also able to see insulin (6 kDa) diffuse through the pores. However, the fluid was not a flow through system as in the microneedle. Thus, large proteins should be selectively excluded from permeating the microdialysis membrane since they cannot fit inside the small channels. Smaller proteins may be able to permeate the membrane but their motion should be retarded. Even if some protein does permeate the membrane, the concentration in the dialysis fluid should be at a

Fig. 9. (Left) Fluorescent dye emerging from pores on the needle tip. (Right) Fluorescent dye in equilibrium with a water droplet on the needle. The pores can be seen as small dots on the needle surface.
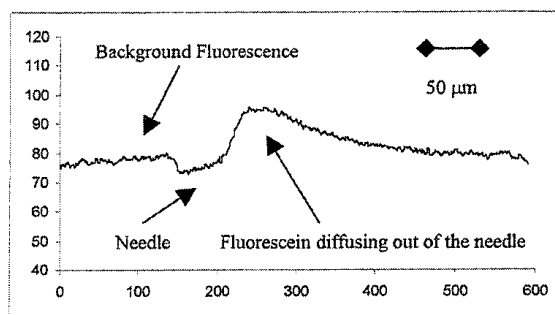
Fig. 10. Fluorescence signal as dye diffuses out of a needle. The low signal indicates the side of the needle, and the dye flux is from left to right starting at approximately 210 on the x axis. The fluorescence signal is directly proportional to dye concentration in solution.

significantly lower concentration than in the bulk which will extend the performance lifetime of a biosensor. Permeable polysilicon membranes should have faster mass transfer rates and better filtration capabilities than diffusion channels since they are a very thin membrane will smaller pores, and a grater surface area for diffusion to occur over. The smaller pore sizes will more effectively retard large MW proteins. However, the thin membranes also make them extremely fragile, which may preclude them from being effectively used. Future work will focus on determining how larger molecules such as serum proteins permeate a microdialysis membrane to allow optimization of the design. This will allow a balance between mass transfer rates and filtering effectivness of a membrane.

Based on estimates, it will take about 15 seconds for analytes to equilibrate through the microdialysis needle. At a flow rate of 1 nl/s, less than 100 µl of dialysis fluid will be consumed after 24 hours of continuous operation. A flow rate of 1 nl/s will ensure that the dialysis fluid will equilibrate with interstitial fluid. Figures (7) and (8) indicate that at 1 nl/s the fluid only needs to travel only 300 µm down the passage before it is at equilibrium. Since the flow passages designed are 4 mm long, the fluid should have more than sufficient residence time to come to equilibrium.

Since the needle volume is only 2.6 nl, a flow rate of 1 nl/s could be very quickly moved onto a planar electrochemical sensor and continuous real time monitoring could be performed, with only a few seconds delay between the fluid sampling and sensing. Microdialysis microneedles are an attractive first step in maximizing the efficiency of portable medical monitors by decreasing size, patient discomfort, fluid volumes, and therefore energy consumed by the device.

ACKNOWLEDGMENT

REFERENCES

[1] N. Talbot and A. P. Pisano., "Polymolding: two wafer micromolding of closed flow passages for microneedles and microfluidic devices," Proceedings 1998 Solid State Sensor and Actuator Workshop, Hilton Head S.C., pp. 265-268, June 1998.
[2] L. Lin, A. P. Pisano, and R. S. Muller, "Silicon processed microneedles," Solid State Sensors and Actuator Conference, Transducers '93, Japan, pp. 237-240, 1993.
[3] S. Henry, D. V. McAllister, M. Allen, and M. Prausnitz, "Micromachined needles for the transdermal delivery of drugs," Proceedings of the IEEE Eleventh Annual International Workshop on MEMS, Heidelberg, Germany, pp. 494-498, January 1998.
[4] J. Brazzle, I. Papautsky, and A.B. Frazier, "Micromachined needle arrays for drug delivery or fluid extraction." IEEE Engineering in Medicine and Biology Magazine, vol.18, (no.6), IEEE, pp.53-58, Nov.-Dec. 1999.
[5] K. Lebouitz, "MEMS microshells for microneedles, microscale fluid visualization, and vacuum packaging of microdevices," University of California at Berkeley, Ph.D. Thesis, 1998.
[6] M. Lambrechts and W. Sansen, Biosensors: microelectrolchemical devices, New York, NY, The Institute of Physics Publishing, 1992.
[7] B. C. Towe and V. B. Pizziconi, "A microflow amperometric glucose biosensor," Biosensors and Bioelectronics, Vol. 12 (no. 9-10), Elsevier, pp. 893-899, 1997.
[8] T. A. Desai, W. H. Chu, J. K. Tu, G. M. Beattie, A. Hayek, and M. Ferrari, "Microfabricated immunoisolating biocapsules," Biotechnology and Bioengineering, Vol 57 (N1), pp 118-120, Jan 1998.
[9] T. A. Desai, D. Hansford, and Ferrari, M. "Characterization of micromachined silicon membranes for immunoisolation and bioseparation applications," Journal of Membrane Science, Vol. 159 (N1-2) pp. 221-231, Jul. 1, 1999.
[10] E. M. Renkin, "Filtration, Diffusion, and molecular sieving through porous cellulose membranes," Journal of General Physiology, Vol 41, pp. 225-243, 1954.